

SEM Studies on Vessels in Ferns. 20. Hawaiian Hymenophyllaceae¹

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ABSTRACT: Tracheary elements of three species (*Mecodium recurvum*, *Vandenboschia devallioides*, and *Callistopteris baldwinii*) (two epiphytic, one terrestrial) representing three genera of Hymenophyllaceae were studied with scanning electron microscopy (SEM). Both roots and rhizomes of all three species possess vessel elements. Wide perforations, an expression of pit (perforation) dimorphism within perforation plates, are prominent, more so than in most other fern families. Monomorphic perforations are also common, as are perforations in which weblike or porose pit membranes are present. Habitats of Hymenophyllaceae are characterized by high humidity with little fluctuation. However, fluctuation in moisture availability within the substrates of Hymenophyllaceae may be related to the abundance of vessels and the distinctiveness of the perforation plates. A peculiarity of hymenophyllaceous tracheary elements not hitherto reported in ferns to our knowledge is reported: gaps in the secondary wall pattern at outer surface of cell angles. These gaps take the form of rhomboidal depressions or a continuous depressed strip.

FERN XYLEM AS studied by scanning electron microscopy (SEM) has been the objective of 20 of our papers, counting this one. This series has been more extensive than we originally envisioned because vessels have proved to be more widespread in ferns than had been expected on the basis of data obtained by earlier workers who used light microscopy. We wished to study genera in which vessels had been claimed by various authors on the basis of light microscopy: *Pteridium* (Carlquist and Schneider 1997a) and *Marsilea* (Schneider and Carlquist 2000b). We also wished to study ferns in which specialized end walls on tracheary elements had been reported by White (1962). In all of these genera, the end walls proved to be perforation plates when studied with SEM; the genera in this category included *Phlebodium* (Schneider and Carlquist 1997), *Astroblepis* (Carlquist and Schneider 1997b), and *Wood-*

sia (Carlquist et al. 1997, Carlquist and Schneider 1998a, Schneider and Carlquist 1998). We extended our SEM studies to genera and families in which tracheary elements showed little or no differentiation of end walls from lateral walls on the basis of light microscopy. All genera we examined in this category proved to have vessels, with the exception of *Botrychium*. Our criteria for presence of vessels was absence of pit membranes on at least groups of pits, if not the entirety of a facet, in macerations (plus sections in a few cases). We frequently observed porose or weblike pit membranes at upper and lower ends of perforation plates in which perforations clear of pit membranes were seen. Incipient perforation plates, in which several to numerous pits with porose pit membranes were observed, are present in these genera.

In this series of papers, we have attempted to select genera from all major fern families, including Psilotaceae (Schneider and Carlquist 2000a). This paper completes this survey. The presence of perforation plates did not prove to be related to the phylogenetic position of fern families, whether the position is generated on the basis of morphological

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studies (Smith 1995) or molecular studies (Pryer et al. 1995). We were also concerned with comparisons between nature of perforation plates and the nature of the habitats (highly seasonal versus uniformly moist) for particular genera. Most of the genera with appreciable difference in perforations of end walls from pits of lateral walls (as judged by perforations larger than pits of lateral walls) were from highly seasonal habitats.

Hymenophyllaceae are notable for occupying almost perpetually moist understory habitats. However, Hymenophyllaceae do require some movement of air and thus have not been successfully cultivated in closed containers. Hymenophyllaceae may be more tolerant of desiccation than has been appreciated. On Antipodes Island (New Zealand), several species of Hymenophyllaceae grow without any cover by trees or shrubs on a plateau that during the week-long visit by S.C. in November 1989 experienced no rainfall and extended sunny periods (S.C., unpubl. observations). Many Hymenophyllaceae are epiphytic, but epiphytes experience fluctuations of moisture availability even in quite rainy and shady localities. In this study, *Mecodium recurvum* (Gaud.) Copel. is a relatively diminutive epiphyte, whereas *Vandenboschia davallioides* (Gaud.) Copel. is a more robust epiphyte; both occur widely in wet Hawaiian forests (Hillebrand 1888). *Callistopteris baldwinii* (Eaton) Copel., in contrast, is terrestrial and is found under trees in cloud forest of O'ahu (Hillebrand 1888).

MATERIALS AND METHODS

Living specimens of the three species studied were collected in the field. Portions were preserved in 50% aqueous ethanol. Collection data are as follows: *Callistopteris baldwinii* and *Vandenboschia davallioides* were collected in the wild in the Lyon Arboretum of the University of Hawai'i (Mānoa Valley, O'ahu) on 29 March 1999 by Ray Baker. Voucher specimens of each are housed at LA. The material of *Mecodium*

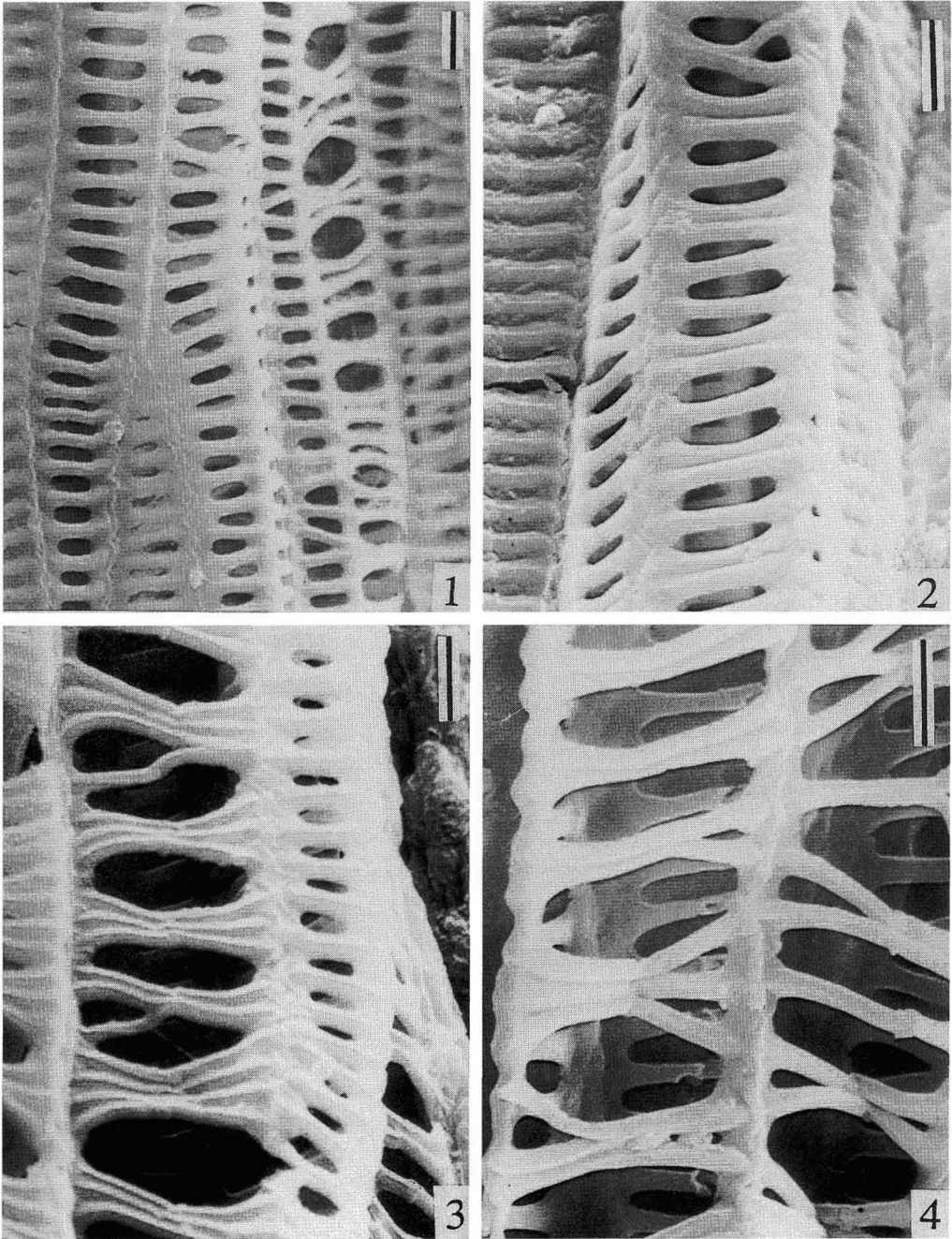
recurvum was collected by E.L.S. near the Thurston Lava Tube, Hawai'i Volcanoes National Park, Hawai'i.

The stems of *Callistopteris baldwinii* are short and about 1 cm thick; roots are relatively thick (ca. 1 mm). *Mecodium recurvum* has slender rhizomes (ca. 1 mm in diameter) and very slender, wiry roots. In *Vandenboschia davallioides*, the rhizomes are about 2 mm in diameter, and roots are about 0.5 mm in diameter. The very slender roots of *Mecodium recurvum* provided technical difficulties because they are rich in fibers, with relatively few tracheary elements. Macerations were prepared by means of Jeffrey's Fluid (Johansen 1940) and stored in 50% ethanol. Macerated portions were spread onto the surfaces of aluminum stubs, air-dried, sputter-coated, and examined with an SEM (Bausch and Lomb Nanolab).

RESULTS

Callistopteris baldwinii

Tracheary elements of roots (Figures 1–4) have numerous and well-defined perforation plates. In Figure 1, a few facets with intact pit membranes are present, but most facets are perforation plates with all perforations alike. At right, but not far right, in Figure 1 is a perforation plate with dimorphic perforations: about seven wider perforations, oval, alternating with narrower perforations and intact pits. Dimorphism of a less pronounced sort can be seen in a prominent perforation plate near center in Figure 2. Most perforations are well developed, but intercalated between these perforations are five narrow pits that are so narrow that they are mere lines and lack perceptible pit membranes. Facets at left and at right in Figure 2 are composed of pits with intact membranes. In Figure 3, a perforation plate that has resulted from marked dimorphism is shown. Wide perforations alternate with several linear, extremely narrow pits. To the right of this perforation plate is a narrow facet that is composed of monomorphic perforations. In



FIGURES 1–4. Tracheary elements of roots of *Callistopteris baldwinii*. 1. Several perforation plates, all but one with monomorphic perforations. 2. Tracheary element with perforation plate (center) with dimorphic perforations and (left and right) elements with scalariform pitted walls. 3. Perforation plate with very wide perforations alternating with groups of very narrow, linear pits. 4. Portions of two facets with dimorphic perforations; note large area of wall devoted to perforations. Scales in all figures = 5 μm .

Figure 4, portions of two perforation plates are illustrated. These perforation plates both show pit dimorphism in which each wide perforation typically alternates with a narrow, nearly occluded perforation. The large perforations in Figure 3 and Figure 4 potentially offer an excellent mechanism for enhanced conduction.

Some perforations (or pits transitional to perforations) in roots of *Callistopteris baldwinii* retain pit membranes. Three porose pit membranes are shown in Figure 5. In Figure 6, the perforations contain more fragmentary webs; some of the pit membrane portions are porose.

A similar array of pit membrane remnants can be seen in perforation plates (or incipient perforation plates) of rhizomes of *Callistopteris baldwinii* (Figures 7, 8). Strandlike and weblike remnants are evident in the perforations of Figure 7, whereas in Figure 8, perforations contain more nearly intact pit membranes, or else none at all, and there are prominent circular to oval pores in the membranes of Figure 8.

Larger portions of rhizome tracheary elements of *Callistopteris baldwinii* are illustrated in Figures 9–12. The tracheary element in Figure 9 shows a facet, right, in which pit dimorphism forms the basis for the perforations. The other facets are little-altered lateral walls. In Figure 10, a distinctive blunt-tipped tracheary element illustrates a variety of perforation sizes and shapes. On the center facet, some very wide perforations alternate with very narrow pits and perforations. The facet at right in this element is virtually uniform in width, however. Figure 11 illustrates apparent transitions between perforation plates and pitted lateral walls: such a transition occurs at upper left, and on the facet at left of the tracheary element at right. In Figure 12, an example of pronounced pit dimorphism in a perforation plate is illustrated. Such a perforation plate looks much like perforation plates with few bars and wide perforations often illustrated in angiosperms because of the exceptionally wide perforations, but pit dimorphism as illustrated here has not been reported in angiosperms.

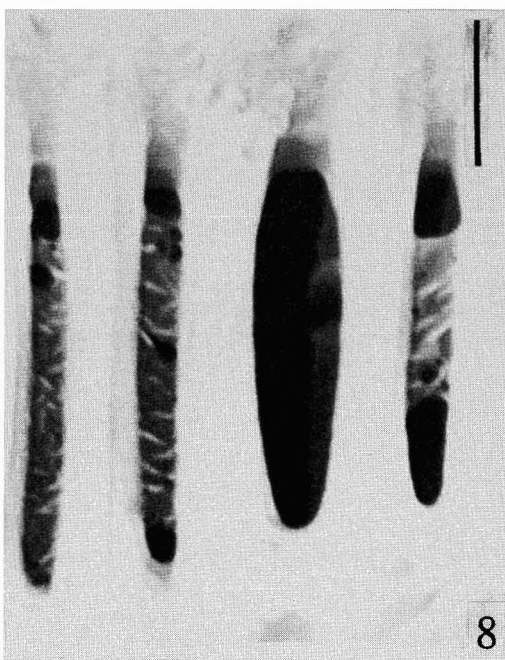
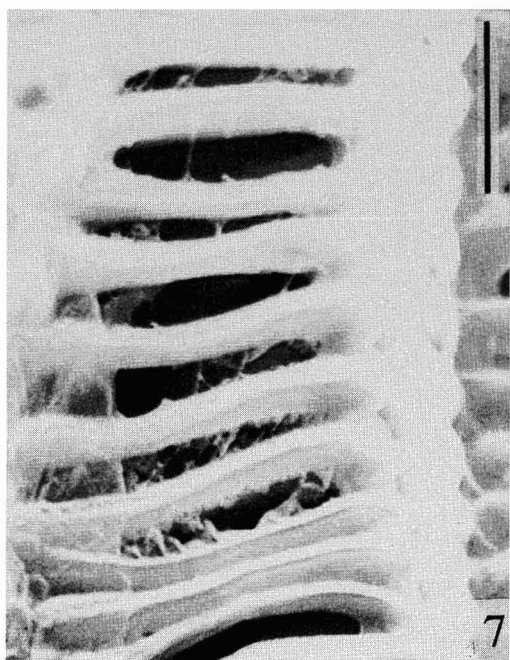
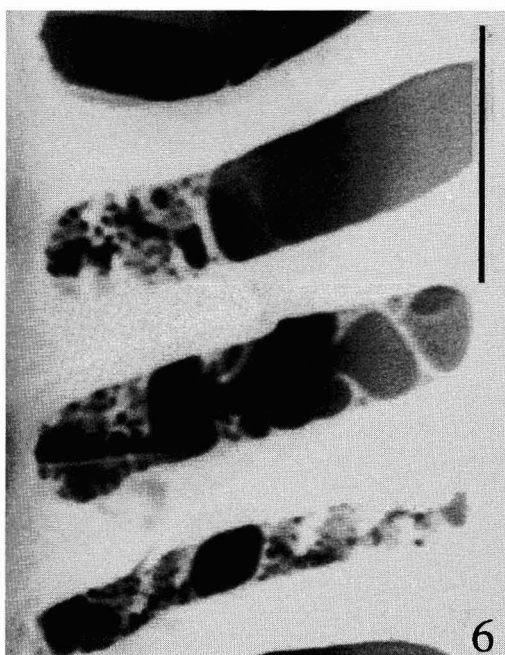
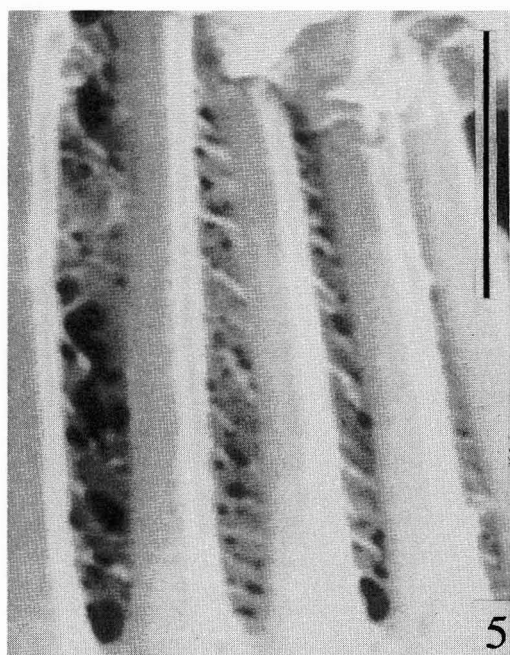
Mecodium recurvum

Because of the very slender, wiry nature of roots in this species, few tracheary elements were recovered in our macerations. A root tracheary element tip is shown in Figure 13. The perforations are notably diverse in shape, with forked and fused bars between some perforations.

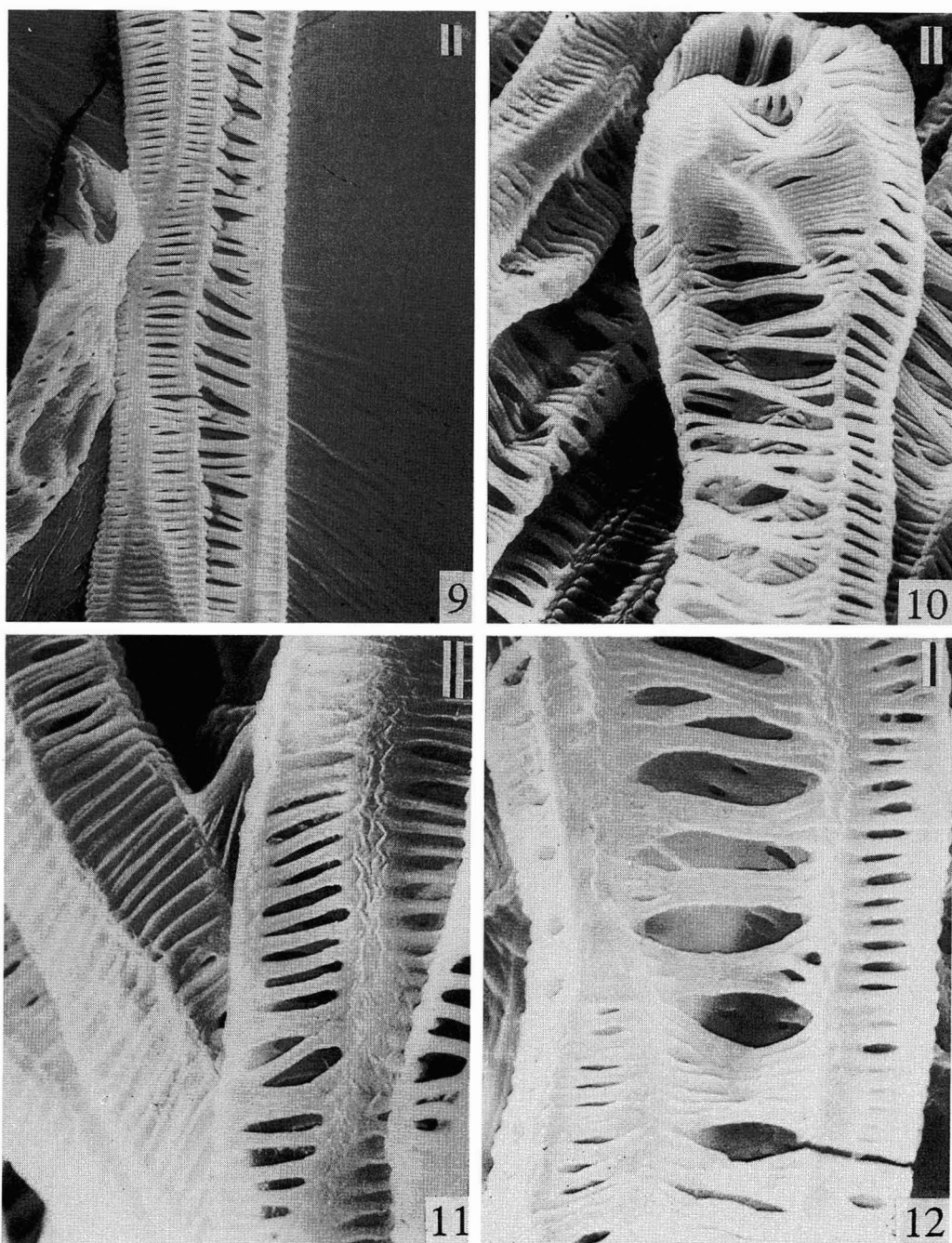
Rhizome tracheary elements of *M. recurvum* are illustrated in Figures 14–17. The element portion in Figure 14 is taken from near the cell tip. This portion clearly shows two facets in which there are perforation plates, above, and intact pitting, below. Although scalariform pitting and perforations characterize most tracheary elements in *M. recurvum* rhizomes (Figures 14, 16, 17), a few tracheary elements with circular to oval pits (with prominent borders), as in Figure 15, were also observed. The tracheary element portions illustrated in Figures 16 and 17 were selected to show pit membranes that appear to have thin and thick portions. The facet at right in Figure 16 shows striate pit membranes; these may represent an artifact, although only some pit membranes in a preparation develop these striations. The center facet in Figure 16 illustrates pit membranes that appear very thin in places. The facet illustrated in Figure 17 similarly suggests unevenness in thickness of pit membranes (one pit, above center of photograph, probably lacks a pit membrane because of artifact formation).

Vandenboschia davallioides

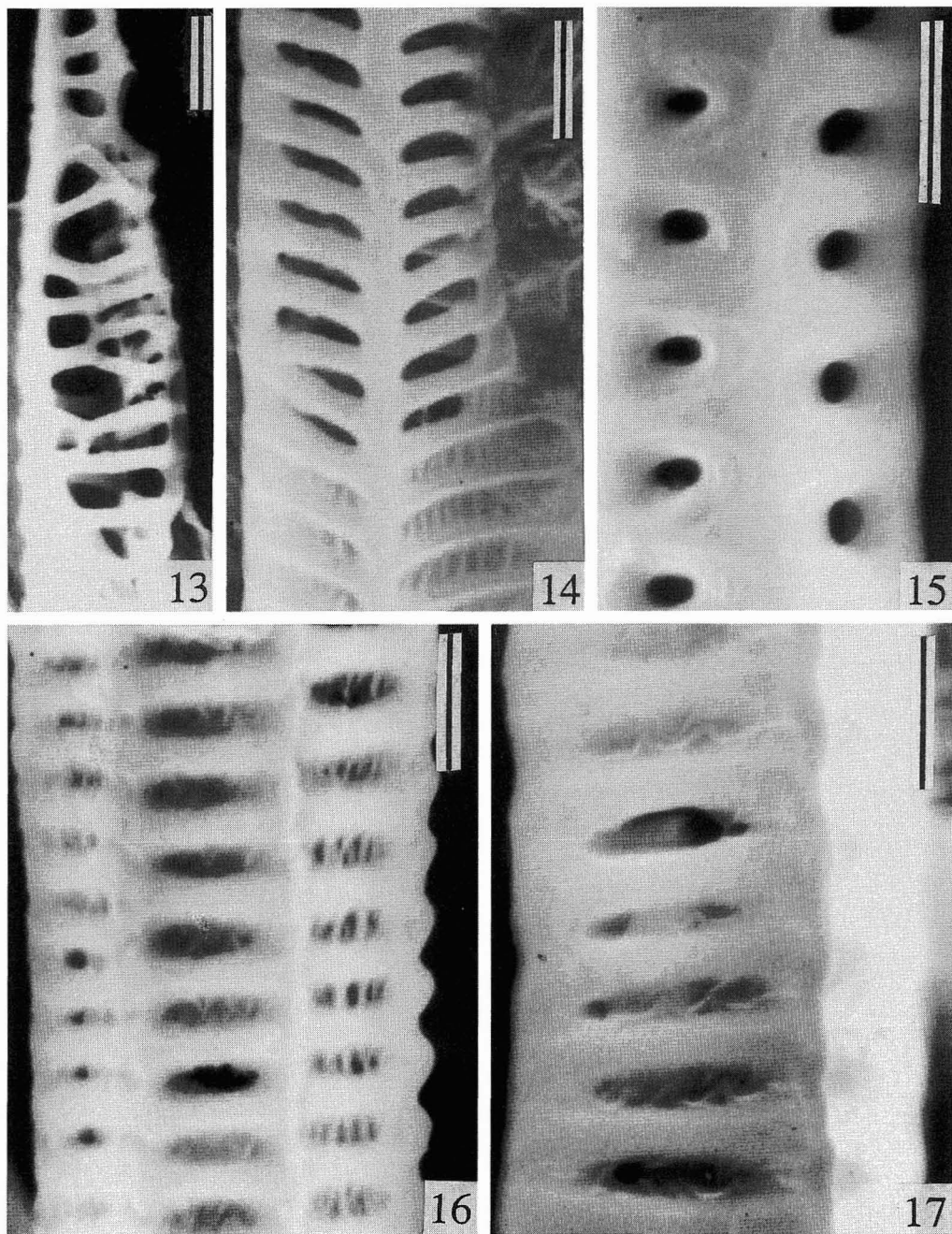
Portions of tracheary elements from roots of *V. davallioides* are illustrated in Figures 18–21. The element in Figure 18 illustrates notably wide monomorphic perforations. The tracheary element in Figure 19 also illustrates perforation plates. However, the perforations in the facet at extreme left in Figure 19 are almost square because of the narrowness of the facet; this facet could nevertheless be termed scalariform. The wide prominent facet at the center of Figure 19 illustrates forked and fused bars, a feature



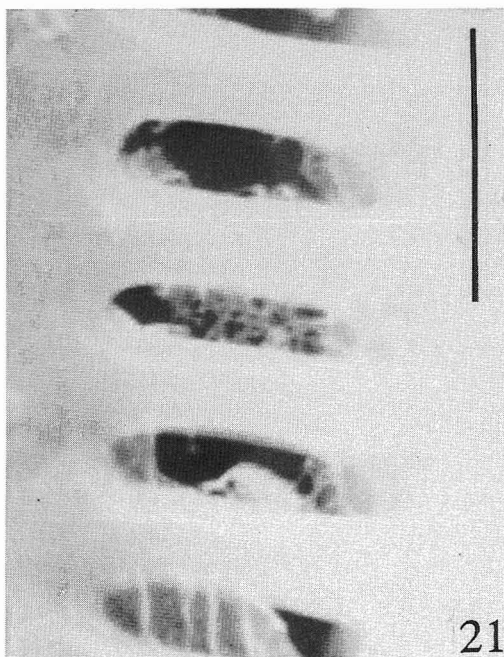
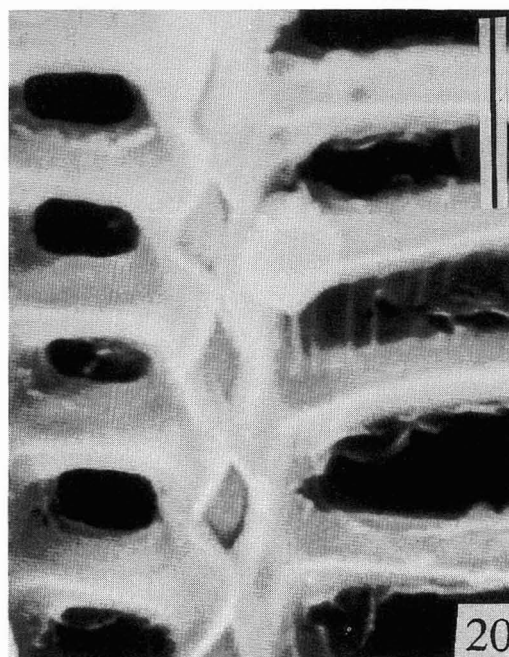
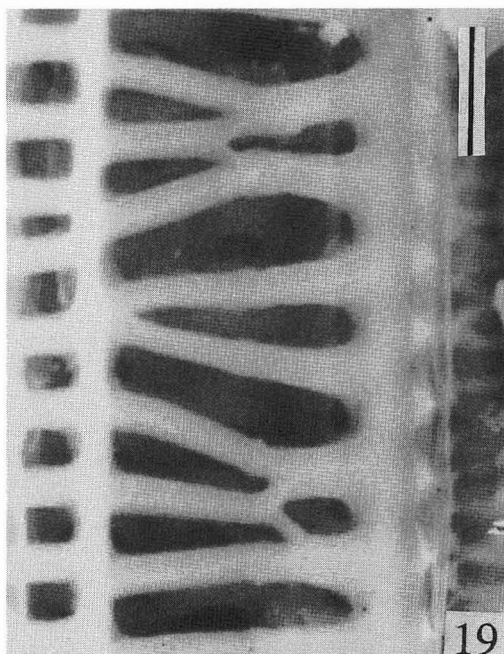
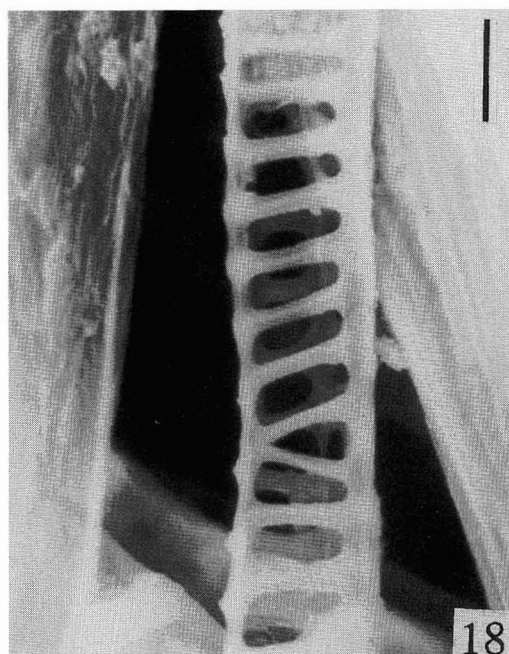
FIGURES 5–8. Incipient perforations from tracheary elements of roots (Figures 5, 6) and rhizomes (Figures 7, 8) of *Callistopteris baldwinii*. 5. Porose pit membranes. 6. Perforations with weblike and threadlike pit membrane remnants; some remnants porose. 7. Perforations with threadlike pit membrane remnants (membranous covering of element at lower left of photo is an artifact). 8. Perforation plate portion with one wide, membrane-free perforation and three perforations with pores of various sizes. Scales in all figures = 5 μm .



FIGURES 9–12. Tracheary elements from rhizome of *Callistopteris baldwinii*. 9. Element with scalariform perforation plates and pitted lateral walls; the plate to the right of center of the element has dimorphism in perforation plates. 10. Tip of a tracheary element with diverse facets and diverse perforation types. 11. Tracheary elements in which the facets shown have transitions from perforation plates to pitted wall surfaces. 12. Element facet (center) with notably wide perforations, representing dimorphism of the perforations. Scales for all figures = 5 μ m.



FIGURES 13–17. Tracheary elements from root (Figure 13) and rhizomes (Figures 14–17) of *Mecodium recurvum*. 13. Element portion near cell tip with diverse sizes and shapes of perforations. 14. Two facets near cell tip; wall portions above are perforations, whereas wall portions in lower third of photograph are pitted wall surfaces. 15. Two facets of slender element; the perforations are nearly circular and have prominent borders. 16. Three facets of a tracheary element, all of which bear pitting; pits of the central facet appear to have thin, weblike membranes. 17. Wall facet with rather thin, weblike pit membranes; lack of a membrane in one pit, a third of the distance from top of photo, is probably an artifact. Scales in all figures = 5 μ m.



FIGURES 18–21. Tracheary elements from roots of *Vandenboschia davallioides*. 18. Slender element with relatively large perforations. 19. Wall facet (center) with forking and fusing of bars between perforations. 20. Angle (just left of center) between two facets (left and right); diamond-shaped depressions on the cell angle parallel the rows of pits. 21. Wall portion with four pits; the pit just above center has a porose pit membrane remnant (the other pits may lack at least portions of membranes because of tearing). Scales for all figures = 5 μm .

not often seen in tracheary elements of ferns. The tracheary element of Figure 20 is shown to highlight a feature we observed not only in *V. davallioides*, but also in other Hymenophyllaceae: lacunae on the outer cell surface at cell angles within the secondary wall pattern (see also Figures 2, 7, 11). In Figure 20, these lacunae are rhomboidal in shape. We have not seen reports of this phenomenon in literature on fern tracheids, nor have we observed it in fern families other than Hymenophyllaceae. The pits shown in Figure 20 seem to show torn remnants of pit membranes, indicating artifact formation, but the secondary wall pattern at the cell angle is not the result of artifact formation. The facet of Figure 21 is presented to illustrate a porose pit membrane remnant (near center); this pit membrane has little tearing; some tearing is evident in the other pit membranes shown.

Rhizome tracheary elements of *V. davallioides* (Figures 22–25) show features that characterize other Hymenophyllaceae and thus enhance our understanding of element structure in the family. In Figure 22, there are, from left to right, a perforation plate with pit (or perforation) dimorphism; a facet with intact pit membranes; a facet with monomorphic perforations; and (on another element) several narrow facets. In Figure 23, very prominent perforations of the dimorphic sort are illustrated; two or three narrow pits intervene between these large perforations. At the far right in Figure 23 are two facets with monomorphic perforations. Pit membranes transitional to perforations are illustrated in Figure 24: the pit membranes are weblike, threadlike, or porose. Figure 25 shows a perforation plate that combines several features: dimorphism of pits, porose narrow pit membranes, vestigial threads in some wider perforations, and large oval or elliptical openings in some pit membranes.

CONCLUSIONS

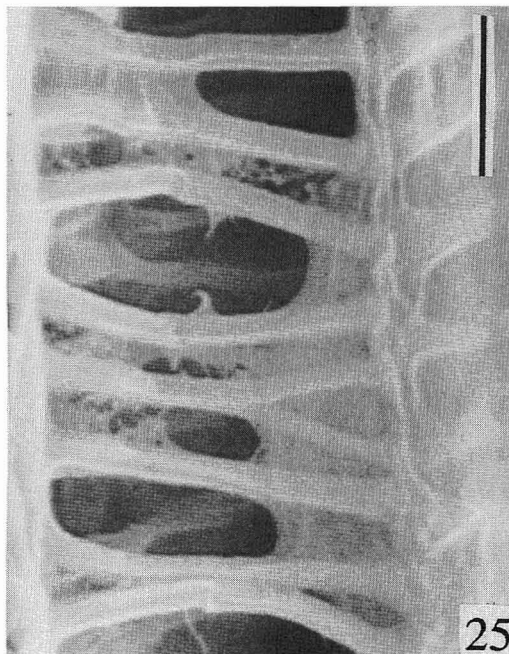
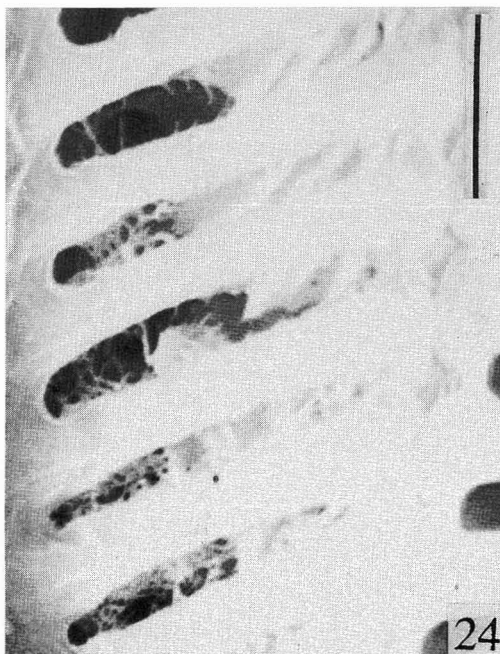
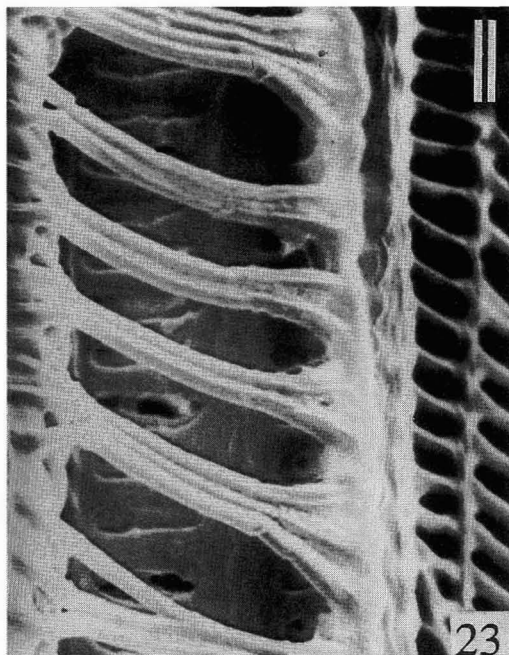
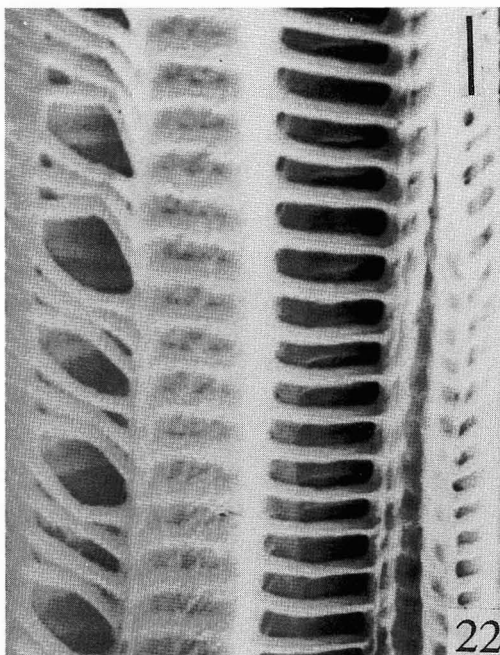
Although Hymenophyllaceae probably do not grow in areas where humidity fluctuates markedly, they do grow in areas where moisture availability fluctuates. This must

be true especially for some epiphytic Hymenophyllaceae, but it may even hold for some terrestrial species such as *Callistopteris baldwinii*. Although there are no quantitative data available on fluctuation in moisture availability in localities of particular species of Hymenophyllaceae, our data on vessels in Hymenophyllaceae indicate prominence in perforation formation, exceeding what we have found in many other families of ferns. For example, perforation dimorphism and perforation size are less in Osmundaceae and Schizaceae (Carlquist and Schneider 1998b) than in Hymenophyllaceae. Caution should be exercised in such comparisons, however, because our survey of ferns has involved a relatively small number of species. We hope that future studies will address in greater depth correlations (or lack of them) between perforation plate morphology and fluctuations in moisture availability. The pit and therefore perforation patterns of tracheary elements of Hymenophyllaceae are basically scalariform, as reported for one species of the family by Bierhorst (1960).

It is interesting that our data suggest that perforations are equally as prominent in roots as in stems of Hymenophyllaceae. A possible explanation is that there is little difference between these two organ systems with regard to water economy in Hymenophyllaceae.

The perforation plates in both roots and stems of Hymenophyllaceae are notable for prominence of perforations. Although dimorphism in pits and perforations within perforation plates has been found in at least six of the fern families we have studied, this phenomenon is strikingly represented in Hymenophyllaceae. The presence of notably wide perforations is equivalent, in its effect on water conduction, to simplification of perforation plates in angiosperms, which have few bars and wide perforations (this has also been achieved in some vessel elements of at least one fern, *Pteridium* [Carlquist and Schneider 1997a]).

Tracheary elements of all Hymenophyllaceae studied show well the phenomenon of incipient or partial perforations in which pit membranes are porose, weblike, threadlike, or contain large holes. This feature, often



FIGURES 22–25. Tracheary elements from rhizomes of *Vandemboschia davallioides*. 22. Facets (from left to right) are a perforation plate with dimorphic perforations; a pitted lateral wall; and a perforation plate with monomorphic perforations. 23. Exceptionally wide perforations related to perforation dimorphism; two rows of monomorphic perforations at far right. 24. Incipient perforations with threadlike and weblike pit membrane remnants. 25. A perforation plate portion with perforation dimorphism, porose pit membranes, threadlike pit membrane remnants, and large elliptical holes in pit membranes. Scales in all figures = 5 μm .

observed in fern tracheary elements at the transitions between perforation plates and pitting, underlines the incomplete differentiation between perforation plates and lateral wall pitting in ferns as compared with angiosperms. This incomplete differentiation may not represent retention of an intermediate stage in vessel evolution, because ferns are relatively ancient, and sufficient time has been available in ferns for differentiation between perforations and pitting to have developed. Possibly the pressure for complete removal of pit membranes is less than in most angiosperms. If so, this could be related to slower conductive streams in fern tracheary elements as compared with tracheary elements of angiosperms. Other possibilities include the potentially greater strength of a scalariform perforation plate compared with a simple one; or the ability of a scalariform perforation plate to prevent spread of air bubbles from one vessel element to another. Data are lacking on all of these possible explanations, but the widespread occurrence of vessels in ferns poses questions that need responses.

A distinctive feature is reported here for the first time for tracheary elements in ferns, as far as we know: depressions in the secondary wall at the cell angles. This takes the form of a depressed strip or a series of rhomboidal depressions. We can at present offer no developmental or physiological explanations for this phenomenon.

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